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**Chemical enhancement of footwear impressions in blood deposited on fabric –
Evaluating the use of alginate casting materials followed by chemical enhancement**

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ABSTRACT

Most footwear marks made in blood on a surface such as fabric tend to be enhanced *in situ* rather than physically recovered using a lifting technique prior to enhancement. This work reports on the use of an alginate material to recover the impressed footwear marks made in blood and deposited on a range of fabric types and colours. The lifted marks were then enhanced using acid black 1 and leuco crystal violet with excellent results.

This presents a new method for the lifting and recovery of blood impressions *in situ* from crime scene followed by subsequent mark enhancement of the lifted impression.

Keywords: footwear impressions, blood, fabric, amino acid, alginate

INTRODUCTION

Alginate impression materials are primarily used for dental or bite mark casts because of their ease of use, low cost and good patient acceptability [1]. There are a number of different alginate materials produced by different companies with different characteristics and factors such as setting times. [1-3]. The materials are generally supplied as powders which are mixed with water prior to application and setting. These powders mainly consist of sodium or potassium salts of alginic acid (11-16%), gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) (11-17%), trisodium phosphate (1-3%) and inert fillers (65-75%) [4].

Little research has been reported in relation to the application of alginate materials on the recovery of blood impressions. Adair [5] investigated the lifting of footwear impressions made in blood both before enhancement and after enhancement with leuco crystal violet (LCV) and recovered marks from concrete, fabric and human skin using Mikrosil[®], Polyvinylsiloxane (PVS), dental stone, and alginate. Only alginates yielded successful enhancement on all of the surfaces tested. Furthermore, the alginate cast could be treated with LCV after the mark had been recovered, facilitating further enhancement of the mark. Furthermore the original blood impression remained largely unchanged. The alginate casting should be photographed as soon as possible as alginates tend to shrink, resulting in the deterioration of the lifted impression [6]. Wiesner *et al.* [7] compared a number of alginates supplied from different companies including GC, Dentsply and Heraeus Kulzer. The study concluded that Aroma Dust Fine III from GC gave the best results when post-treated with acid black 1 (AB1) rather than LCV while post-treatment with 1,8-diazafluoren-9-one (DFO) did not provide any enhancement. A possible mechanism posited for the reaction of alginate with blood was that the water partially dissolved the blood which became biologically tied to the alginate and retained the fine detail [7].

To date, little research work has been reported on the use of alginate casting materials for the recovery of blood impressions deposited on fabrics.

MATERIALS AND METHODS

Deposition of the footwear impressions and preparation of the test marks

It can be argued that robust comparisons of footwear enhancement techniques can only be made if the test footwear impressions have been prepared in the same manner where these factors have been controlled in each case [8]. Variables introduced during the preparation of test footwear impressions include the pressure of the footwear sole on the receiving surface as the footwear impression is made. Other influencing factors on the quality of the mark include the amount and composition of blood on the footwear sole prior to being transferred to the receiving surface and the actual amount of contaminant transferred to the substrate.

The objective of this work was to compare the ability of various alginate casting materials to recover a footwear mark made in blood from a fabric surface, rather than mimic operational conditions normally encountered. Only when repeatability of the quality of the footwear impression produced could be controlled (such that there was no variation from mark to mark) could a direct comparison of the various alginates be reliably achieved. In this work the pressure applied to the receiving surface by the blood contaminated footwear was precisely controlled using a rig developed and calibrated for that purpose, as shown in figure 1. The device was calibrated to repeatably deliver a force comparable with the average force used in a stamping action as determined through trials conducted with live volunteers (3500 Newtons).

The application of blood to the footwear sole and the subsequent transfer of blood to a substrate are challenging to control during experimental trials. Stepping into a pool of blood followed by stepping onto the fabric resulted in a heavy blood-stained and overloaded footwear impression. The following method however yielded reasonably weak and reproducible bloody impressions from mark to mark. A tray measuring 0.33 x 0.23 x 0.06 m was lined with two Kimberley[®] blue double ply tissues covering the whole base. 50 mL of swine blood was poured over the tissues. The tray was then pushed against the sole of the footwear attached to the rig in a walking motion. The same motion was repeated twice on clean tissues to remove excess blood before releasing the foot onto the fabric. Five individual repeat footwear marks were prepared as described for all tests undertaken. All impressions were allowed to age for seven days before lifting and subsequent enhancement.



Figure 1 – Semi-Automated Stamping Device

For practicality, an ageing time of seven days was chosen. This was done in order to reflect, to some degree, the number of days which commonly elapse between the collection of the item and subsequent chemical processing (as opposed to biological or DNA analysis) of the footwear mark in laboratories during casework. Preliminary work was carried out by the authors where marks were aged for a month and no deterioration of the footwear impression recovered using the alginate and followed by subsequent chemical enhancement was observed. Photography of all impressions was performed under white light immediately after the impression was prepared, after seven days and then after chemical treatment.

Chemical Formulations

The chemical reagents and fabrics utilised in the study are listed in table 1.

Table 1 - List of Chemicals and Fabrics

Peroxidase Reagents and Fabric				
Chemical Name	Alternative Chemical Name	Chemical Supplier	Fabric	Fabric Supplier
Acid Black 1 (AB1)	Amido Black 10B	BVDA	White Cotton (WC) [CD13]	WBL Whaleys Bradford Ltd.
Leucocrystal Violet (LCV)	4,4',4''-Methylidynetris(<i>N,N</i> -dimethylaniline)	Sigma Aldrich	Black Cotton (BC) [CD13D]	WBL Whaleys Bradford Ltd.
Alginate Aroma Fine Dust III (Normal Set)		GC	White Nylon/Lycra (WNL) [SF28]	WBL Whaleys Bradford Ltd.
Alginate Blueprint Cremix		Dentsply	Black Nylon/Lycra(BNL) [SF27]	WBL Whaleys Bradford Ltd.
Alginate Henry Schein		Henry Schein	Denim	Mandors, Glasgow, UK

Alginate

Preliminary work compared alginates from different companies (Aroma Dust Fine (III) by GC, Blueprint Cremix by Dentsply and Henry Schein alginate) on white cotton and denim. The instructions on the GC alginate packaging suggested mixing one scoop (using a plastic scoop provided by the manufacturers) of product with 20 mL of water. Preliminary worked showed that nine scoops of product with 200 mL of water provided enough material with the best consistency to cover a full footwear mark. As soon as water was added to the powder, the mixture was stirred for 30 seconds and applied quickly with a big plastic spatula on the impression. A Ziploc[®] bag was placed over the alginate and pressure was evenly applied across its surface. After one minute the alginate had solidified enough to remove, though it was left to dry overnight. This technique was carried out on white and black nylon, white and

black cotton and Denim. Five repeat trials were performed for each fabric sample. Each footwear mark produced was enhanced according to the methods presented in table 2.

Acid black 1 (AB1) and leuco crystal violet (LCV) were chosen as examples of a protein stain and a peroxidase reagent respectively. AB1, together with acid violet 17 (AV17) and acid yellow 7 (AY7), are recommended as chemical processes for the enhancement of fingerprints in blood in the United Kingdom [9-12]. LCV has been reported to be an excellent technique for the enhancement of footwear impressions in blood [13-15].

Table 2 - Alginate Enhancement Repeats

Repeat Number	Enhancement method
1	Alginate application only
2	Alginate application followed by AB1
3	AB1 followed by alginate application
4	Alginate application followed by LCV
5	LCV followed by alginate application

Acid Black 1(AB1)

Fixing solution:

23 g of 5-sulfosalicylic acid dihydrate (Acros) was dissolved and stirred in 1 L of distilled water. This was used to fix blood impressions by immersion for a minimum period of 5 minutes when staining with AB1.

Staining solution:

1 g of acid black 1 (BVDA) was stirred for at least 30 minutes in 50 mL acetic acid (Sigma), 250 mL ethanol (Sigma) and 700 mL distilled water. This was used to stain blood impressions by immersion for a minimum period of 3 minutes.

De-staining solution:

A solution of 50 mL acetic acid, 250 mL ethanol and 700 mL distilled water was prepared for de-staining. This was used to wash the excess dye from the background. All items were air dried overnight before photography.

LCV

10 g of 5-sulphosalicylic acid dihydrate (Acros) was dissolved in 500 mL of 3% hydrogen peroxide (VWR). 3.7 g sodium acetate (Sigma) was added to the mixture followed by 1 g of leuco crystal violet (Sigma) and stirred until dissolved completely using a magnetic stirrer. Application was carried out by spraying with an Ecospray[®] unit supplied by Bluestar[®] Forensic, as recommended by the Home Office Scientific Development Branch (HOSDB).

RESULTS AND DISCUSSION

Comparisons of Alginates

The alginate from GC, Aroma Dust Fine III, gave best overall results and produced the best contrast and greatest level of detail for the lifted blood impression, corroborating previous research [7]. Blueprint Cremix from Dentsply produced good quality lifted impressions, however, its use obliterated the original blood impression on the fabric and the blood impression contrast on blue alginate was poor when compared to the impressions on pink alginate from GC. The alginate from Henry Schein gave poor overall results when compared to the other alginates.

During the tests, some seepage of water occurred from the alginate mix into the fabric causing damage to the impression. The ratio of nine scoops of powder to 200mL of water resulted in more water to powder than the suggested ratio by the manufacturer (10 scoops : 200mL water) which could explain the 'wetter' factor. A trial was performed using 10 scoops of powder to 200mL of water, resulting in similar water seepage. One solution to this problem was to mix the water with alginate for a longer period of time, however, because of the fast setting time of alginates this was not always possible.

Although the use of alginates for lifting blood impressions on clothing was investigated by Adair [5] and Wiesner [7], neither study mentioned the fast shrinking rate of alginates. Prompt photography is recommended as the footwear impression detail on the alginate could be compromised due to shrinkage of the alginate as the water is lost. Shrinkage starts to occur as the alginate dries. Photography was carried immediately after lifting the alginate and after drying overnight (16 hours). No difference due to shrinkage was apparent. However, the alginate lift did decrease in size by about a third after 72 hours. Furthermore, it is important to note that the lifted impression is a mirror image of the original impression. The trials shown in table 2 were performed using GC alginate as the best performing alginate.

Post –lifting enhancement

After alginate lifting, the fabrics were treated with the various chemical enhancement reagents. Enhancement with acid yellow 7 (a protein stain), luminol (a heme-reagent) and ninhydrin (an amino acid staining technique) failed to produce any meaningful results for the enhancement of the residual blood impression remaining on the fabric surface, indicating the

interference of alginate with the blood impression. Luminol gave a weak and rapidly fading chemiluminescence.

The best results were obtained by post-enhancement with acid black 1 (figures 2 and 3). Leuco crystal violet applied to the lifted mark also enhanced the impression recovered by the alginate (figure 4), however, the sharpness and colour brightness achieved appeared to be slightly inferior to that obtained using AB1. This difference may be explained by the fact that during treatment with AB1, the fixation process occurs separately and prior to staining. LCV treatment uses the fix and staining in a one-step process. It has been reported that fixing and staining in a one-step process may not be as effective as fixing and staining separately [12]. Separation of the fix from the LCV formulation can decrease the effectiveness of the technique. The reaction of blood with LCV results in the oxidation of LCV to crystal violet, a pH indicator which changes to a yellow colour in acidic conditions. Powell [16] investigated the use of a fixing agent before applying the LCV formulation in two different ways. The first method removed the fix from the LCV formulation and fixed separately before applying LCV. However, this resulted in the formulation not being acidic enough to fully dissolve the LCV and the amount of LCV in solution was insufficient for a colour change to occur. The second method involved fixing the impression before applying the LCV formulation incorporating the fix, making the solution too acidic.

The use of alginate lifting followed by chemical enhancement provided an excellent technique for improving visualisation of the mark as the use of chemical techniques alone do not provide the necessary contrast for on dark fabrics and denim. Although, the alginate cast has been reported to leave the impression in blood visually unchanged [5, 7], the water present in the paste seemed to affect the porous fabric. The fabric seemed to remain wet for a prolonged period of time after the alginate. This is revealed in comparison of figures 2b and 4b to figures 2a and 4a. This effect was also observed on the less porous fabric nylon/lycra.

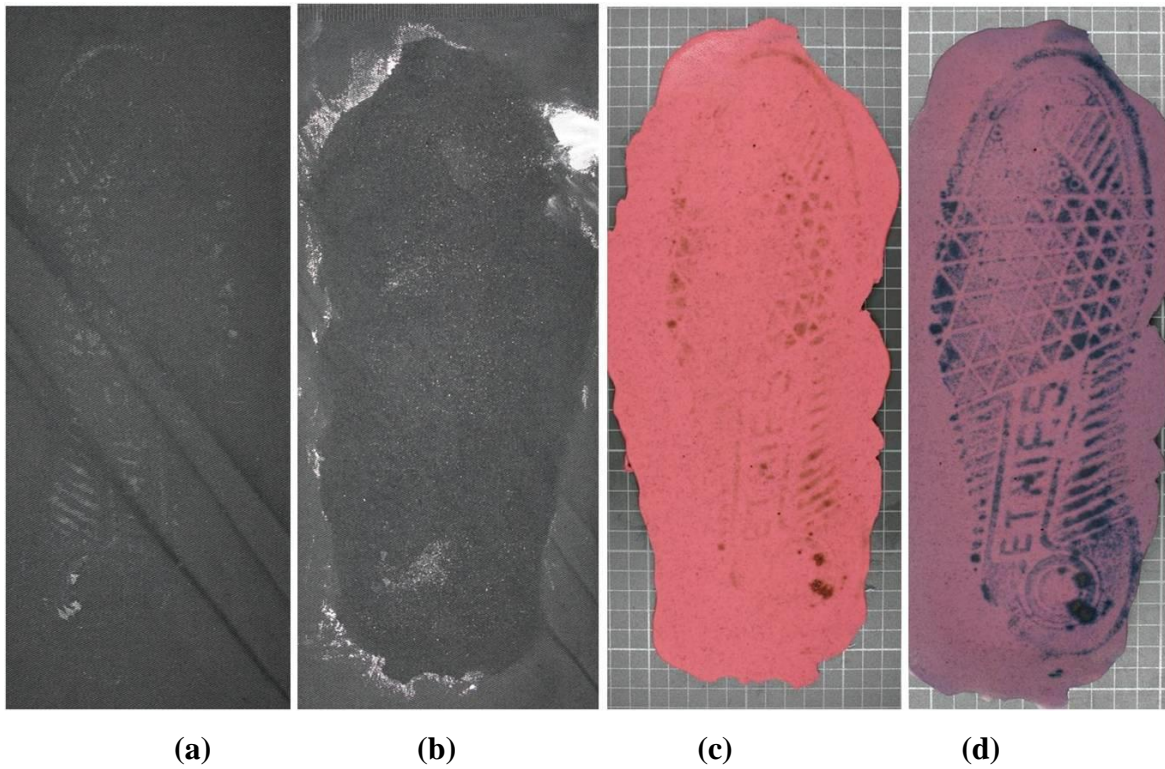


Figure 2 – Enhancement of a footwear impression in blood on black cotton using alginate and AB1: (a) impression in blood before enhancement; (b) impression in blood after lifting alginate; (c) alginate lift; (d) alginate lift enhanced with AB1

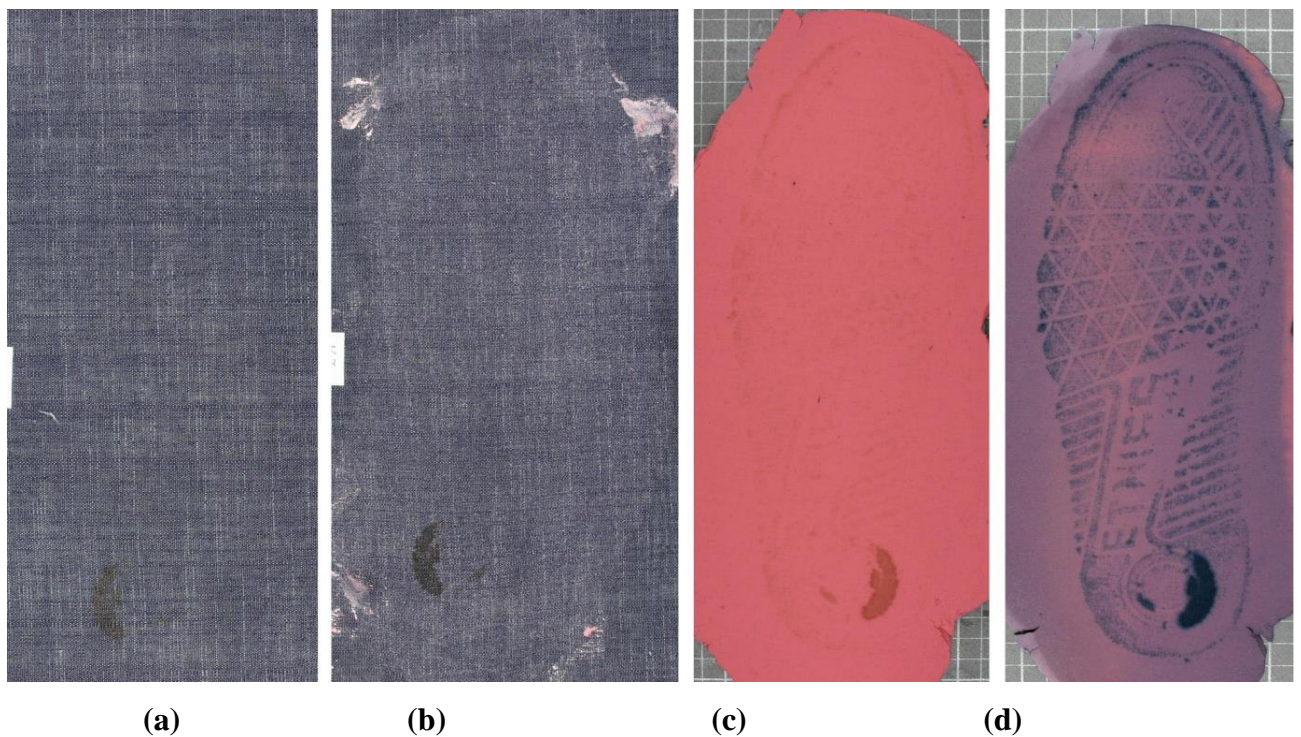


Figure 3 – Enhancement of a footwear impression in blood on denim using alginate and AB1: (a) impression in blood before enhancement; (b) impression in blood after lifting alginate; (c) alginate lift; (d) alginate lift enhanced with AB1

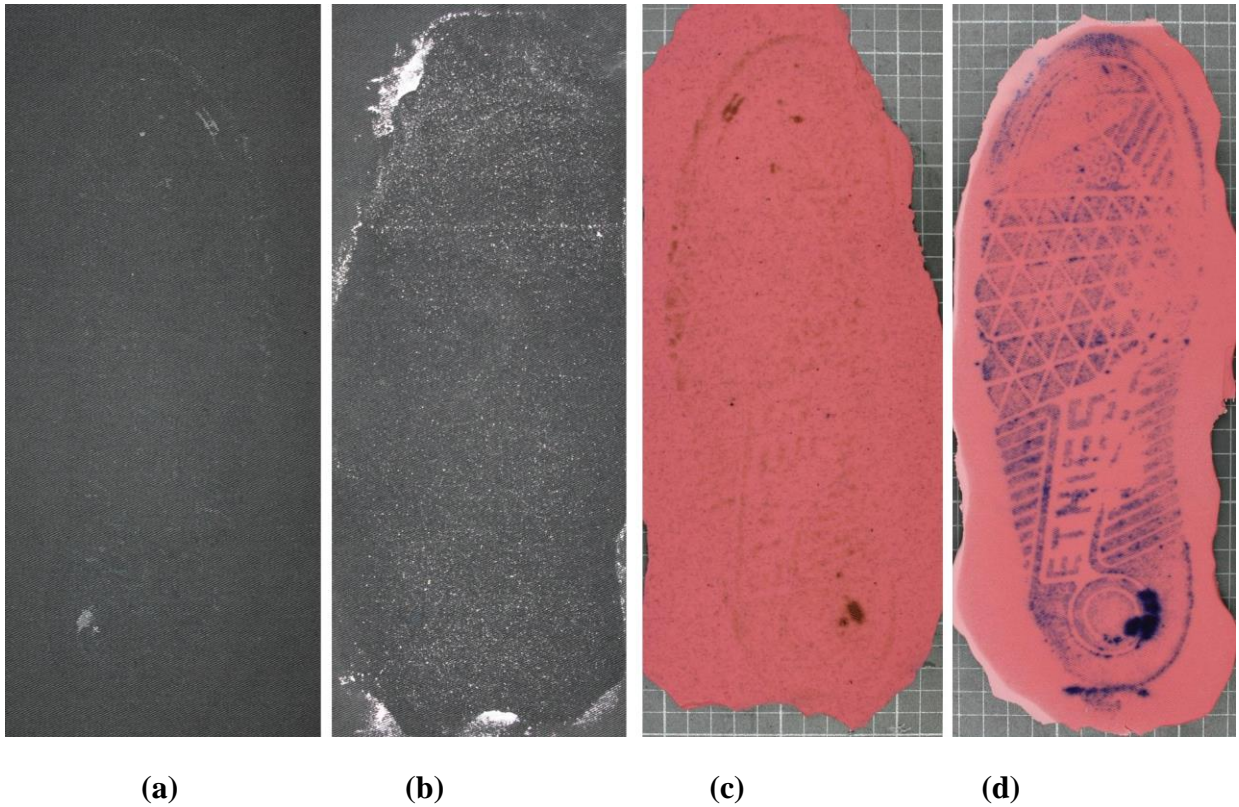


Figure 4 – Enhancement of a footwear impression in blood on black cotton using alginate and LCV: (a) impression in blood before enhancement; (b) impression in blood after lifting alginate; (c) alginate lift; (d) alginate lift enhanced with LCV

CONCLUSION

Alginates are an available technique for the *in situ* recovery of blood impressions. They can easily be prepared and applied at a crime scene if the article cannot be moved to the laboratory. Lifting with alginate followed by acid black 1 or LCV provided good enhancement of all of the impressions on all fabrics including dark fabrics and denim. Nonetheless the use of LCV is not recommended for routine analysis due to health and safety concerns as crystal violet (the product formed from the reaction of blood and LCV) has recently been upgraded to a category 3 carcinogen (MSDS data). Future research will assess the effectiveness of alginate lifting after ageing impressions in blood for longer periods of time followed by enhancement with a number of different stains and other chemical techniques.

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